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ENERGY METABOLISM PATHWAYS OF HYDROTHERMAL VENT ANIMALS: ADAPTATIONS TO A FOOD-RICH AND SULFIDE-RICH DEEP-SEA ENVIRONMENT

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Abstract

The activities of enzymes of the major pathways of energy metabolism (glycolysis, the citric acid cycle, and the electron transport system) were measured in tissues of animals from the deep-sea hydrothermal vent site at 21°N latitude. Enzymic activities of related shallow-living marine animals were assayed for comparison. Vent species studied were the large pogonophoran tube worm, *Riftia pachyptila,* the clam, *Calyptogena magnifica,* the crab *Bythograea thermydron,* the polychaete worm, *Alvinella pompejana,* and an unidentified zoarcid fish. In general, the enzymic activities found in the tissues of the vent animals were qualitatively and quantitatively similar to those of phylogenetically related shallow-living marine species, suggesting that the types of energy metabolism pathways, and the potential flux rates through these pathways, are similar in both groups. The enzymic activities of the vent zoarcid fish were much higher than those of all other deep-sea fishes studied to date. Despite the occurrence in the vent waters of high concentrations of hydrogen sulfide (HS^-), a potent inhibitor of the cytochrome c oxidase system, most of the vent animals possessed cytochrome c oxidase activities comparable to those of related shallow-living species. The cytochrome c oxidase systems of the vent species and shallow-living species so examined were half-inhibited by HS^- concentrations in the nanomolar to micromolar range. The mechanisms by which the vent animals avoid poisoning of respiration by HS^- are discussed. *Calyptogena magnifica* was the only vent species that appeared to have a minimal capacity for aerobic respiration, as judged by extremely low activities of the cytochrome c oxidase system and citrate synthase in its tissues compared to other bivalves. We propose that *C. magnifica* may rely largely on anaerobic pathways of energy metabolism.

Introduction

The unusual water chemistry and biological characteristics of the deep-sea hydrothermal vent habitats may favor a number of adaptations in the energy metabolism pathways of the vent animals. Unlike typical deep-sea regions, the hydrothermal vents have a dense biomass (Spiess *et al.,* 1980) which appears to be supported by primary production by chemolithotrophic bacteria, especially sulfide oxidizing species. These bacteria are free-living in the sea water (Karl *et al.,* 1980; Tuttle *et al.,* 1983) and symbionts of dominant members of the vent fauna, including the large pogonophoran tube worm, *Riftia pachyptila* (Cavanaugh *et al.,* 1981; Felbeck, 1981; Felbeck and Somero, 1982), the clam, *Calyptogena magnifica* (Felbeck *et al.,* 1981; Cavanaugh, 1983), and the unnamed vent mussel (Felbeck *et al.,* 1981). The presence at the vents of a rich food supply, by deep-sea standards, may permit

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a relatively high rate of energy metabolism in the vent animals compared to animals from the typical deep sea. The latter animals may have oxygen consumption rates that are only a few percent of those of related shallow-living species (Childress, 1971, 1975; Smith and Hessler, 1974; Smith, 1978; Torres *et al.,* 1979; Somero *et al.,* 1983), and these extremely low rates of metabolism may reflect adaptations to the low food availability in non-vent deep-sea habitats.

Despite the occurrence of a rich food supply in the vent habitats, however, the presence of high (up to 1 m*M*; Edmond *et al.,* 1982) concentrations of hydrogen sulfide (HS^-) in the vent waters could potentially block the abilities of vent animals to metabolize aerobically at high rates. HS^- is a potent inhibitor of the cytochrome c oxidase (CO) system and, therefore, of aerobic respiration (Hydrogen Sulfide, 1979; Powell and Somero, 1983). Thus it is of interest to determine if the energy metabolism pathways utilized by vent animals include the same types of reactions found in marine animals from habitats with low HS^- concentrations, or if the vent animals are unusually dependent on anaerobic mechanisms of energy metabolism.

The present studies examined several animals thought to be endemic to the deep-sea hydrothermal vents, including *R. pachyptila; C. magnifica;* the brachyuran crab, *Bythograea thermydron;* the polychaete worm, *Alvinella pompejana* (Pompeii worm); and an unidentified fish of the family Zoarcidae. We sought answers to the following questions. First, are the types of aerobic and anaerobic energy metabolism pathways used by the vent animals similar to those found in phylogenetically related shallow-living marine animals? Second, if the vent animals do utilize aerobic respiration, as judged by the presence of the CO system, is this enzyme system less sensitive to poisoning by HS^- than the homologous systems of animals from habitats where HS^- is not present in high concentrations? Third, are the quantities of enzymic activity similar in tissues of vent and non-vent animals? An answer to this question bears directly on the point concerning metabolic rates in the vent animals, since enzymic activity measurements have proven to be a useful means for obtaining estimates of respiration rates of shallow- and deep-living marine animals (cf. Childress and Somero, 1979).

Materials and Methods

The hydrothermal vent animals were collected at the 21°N latitude vent site on the East Pacific Rise (Spiess *et al.,* 1980). Except for the two specimens of the zoarcid fish, which were generously provided by Dr. Harmon Craig following the Pluto Expedition to this site in late 1981, all specimens were collected during the Oasis Expedition in April–May 1982. The fish were frozen (−20°C) shortly after recovery at the surface, and were held frozen until the enzymic activity measurements were made. The enzymes studied in the fish are all known to be stable during freezing (Childress and Somero, 1979). All enzymic activities in the invertebrates were made using tissues from live, freshly collected adult animals. The tissues sampled in the different species are given in the legend to Figure 1. In most cases activities were measured within a few hours of retrieval of the specimens, which were collected at a depth of approximately 2600 m by the DSRV Alvin. The specimens were transported from the collection site to the surface in an insulated box, and were judged in all cases to be in healthy condition. When specimens were maintained alive aboard ship (RV New Horizon), they were held in circulating sea water (2–5°C) at a pressure of 120 atmospheres and used within 2 days. The animals survived for at least several days under these holding conditions.

Live specimens of animals from non-vent habitats were obtained as follows. The stone crab, *Menippe mercenaria,* and the hardshell cockle, *Chione undatella,* were

collected subtidally off La Jolla, California. *Mercenaria mercenaria* (the quahog clam) were collected on the East Coast of the U. S. and purchased from a local seafood supplier. *Solemya reidi*, a gutless bivalve found in sulfide-rich habitats, were collected at depths of approximately 120 m near the Hyperion sewage outfall off Los Angeles, California, using the RV Velero. Specimens of *S. reidi* were maintained in aquaria in the presence of 1 m*M* HS^- until analyzed.

Enzymic activity determinations

For all invertebrates, tissue samples taken from live specimens were homogenized immediately in ice-cold buffer (20 m*M* potassium phosphate, pH 7.4). In the case of the vent species, motion of the ship prevented accurate measurement of tissue weights, so precise dilutions of the tissue samples with homogenization buffer could not be made. Consequently, enzymic activities for the invertebrates are expressed in terms of international units (μmoles substrate converted to product per min) per mg protein in the supernatants. The tissues were homogenized using a Duall-23 ground glass surfaced homogenizer (Kontes Glass Co., Vineland, NJ) driven by hand. The homogenates were centrifuged at 2500 *g* for 10 minutes, and the supernatants were saved and used without further purification for the activity assays. Enzymic activities were measured immediately at a temperature of 20 $\pm$ 0.2°C, using Varian-Techtron 634 or 635 spectrophotometers. The activities presented were all determined at 1 atm pressure. A survey of pressure effects on these enzymes from vent organisms showed that *in situ* pressures (approximately 260 atms) had only minimal effects on activities under our assay conditions using saturating substrate concentrations. Maximal inhibition noted was 7%, and maximal activation was 9%. Thus, the use of 1 atm pressure in these studies is not likely to have led to artifacts.

The enzymic activities in muscle of the vent zoarcid fish were measured in La Jolla, California, using tissue samples from two deep frozen specimens. Muscle samples were removed from the area just behind the operculum and above the lateral line; these samples appeared to be entirely of white muscle. Samples were homogenized in 10 m*M* Tris/HCl buffer (pH 7.5 at 10°C), and the homogenates were centrifuged at 2500 *g* for 10 minutes. Enzymic activities were measured at 10 $\pm$ 0.2°C, and are expressed as international units per g fresh (wet) weight of tissue. This normalization of activity on a fresh weight basis for the fish enzymes was done to enable comparisons to be made with data gathered under identical experimental conditions in studies of other deep- and shallow-living marine fishes (Childress and Somero, 1979; Sullivan and Somero, 1980; Siebenaller and Somero, 1982; Siebenaller *et al.*, 1982).

The following enzymes were studied in some or all of the species: L-lactate dehydrogenase (LDH, EC 1.1.1.27; L-lactate: NAD^+ oxidoreductase); pyruvate kinase (PK, EC 1.7.1.40; ATP: pyruvate phosphotransferase); phosphofructokinase (PFK, EC 2.7.1.11; ATP: D-fructose-6-phosphate 1-phosphotransferase); L-malate dehydrogenase (MDH, EC 1.1.1.37; L-malate: NAD^+ oxidoreductase); citrate synthase (CS, EC 4.1.3.7; citrate: oxaloacetate lyase (CoA-acetylating); and cytochrome c oxidase (CO, EC 1.9.3.1; ferrocytochrome c oxygen oxidoreductase).

Measurements of LDH, PK, MDH, and CS activities were performed following the protocols given in Somero and Childress (1980). PKF activities were measured in an assay medium containing 33 m*M* Tris-acetate buffer (pH 8.0 at 20°C), 2 m*M* Mg-acetate, 2 m*M* ATP, 2 m*M* fructose-6-phosphate, 40 m*M* KCl, 4 m*M* NH_4Cl, 0.16 m*M* NADH, 400 μg of aldolase, 20 μg of triose phosphate isomerase, and 50 μg of glycerol-3-phosphate dehydrogenase, as described by Hand and Somero (1982).

CO activities were measured using the protocol of Yonetani and Ray (1965). The assay solution contained 0.1 *M* potassium phosphate buffer (pH 6.0), 1 m*M* EDTA, and 0.1 m*M* reduced cytochrome c, in a total volume of 2.0 ml. The reaction was followed by recording the decrease in absorbance at 550 nm, using an extinction coefficient for cytochrome c of 18.5 mM^{-1} cm^{-1} (reduced minus oxidized). Reduced cytochrome c (horse heart, Type III, Sigma Chemical Co., St. Louis, Missouri) was prepared as follows. A stock solution of cytochrome c (final concentration of 1 m*M*) was prepared in 10 m*M* potassium phosphate buffer, pH 7.0, containing 1 m*M* EDTA. The buffer stock was saturated with N_2 and stored tightly-capped. The cytochrome c solution was reduced by adding trace amounts of sodium dithionite. A change in solution color from reddish-brown to bright red-orange indicated quantitative reduction of cytochrome c. Excess dithionite and its breakdown products were removed by gel sieving with Sephadex G-25, utilizing the centrifugation method of Helmerhorst and Stokes (1980). Sephadex G-25 was hydrated with distilled water and then equilibrated with 10 m*M* potassium phosphate buffer, pH 7.0, containing 1 m*M* EDTA. Next, 3 ml syringes (tips plugged with glass wool) were filled with hydrated G-25 and placed into conical centrifuge tubes. The syringes were centrifuged for 2 minutes at approximately 1900 *g*. The liquid that collected in the bottom of the tubes was discarded, and the reduced cytochrome c solution was added to the syringes. For a syringe with a 3 ml bed volume, about 0.4 ml of solution can be added per syringe. The syringes were then centrifuged as above, and the liquid at the bottoms of the centrifuge tubes was collected. Prepared in this fashion, the cytochrome c is at least 95% reduced. The rate of autooxidation is only about 1–2% per day when the solution is stored tightly stoppered at 2°C.

In view of the occurrence of CO activities in most of the tissues of the vent animals so examined (Fig. 1), it was important to determine if this enzyme system was resistant to inhibition by HS^- in these species. HS^- concentrations in the micromolar range or below typically are strongly inhibitory of CO (Hydrogen Sulfide, 1979; Powell and Somero, 1983).

Except for *R. pachyptila,* the CO activities were determined using the crude supernatant fractions prepared as described above. For the CO of *R. pachyptila* additional tests were run using partially purified CO prepared by sequential acid precipitation of the enzyme system ("once acid precipitated" and "twice acid precipitated"). In this case, the crude supernatant was titrated to pH 5.6 with cold 1.0 *M* acetic acid and then centrifuged at 2500 *g* for 10 minutes. Four to nine concentrations of HS^- were used to determine each K_i value. The stock solution of HS^- was prepared by dissolving freshly washed crystals of Na_2S in deoxygenated distilled water. The data for CO of *R. pachyptila* are derived from data in Powell and Somero (1983).

Protein concentration measurements

For all of the invertebrate tissues the protein concentration of the supernatant fractions was measured using the technique of Peterson (1977).

Results

Enzymic activities of invertebrates from vent and non-vent habitats

Figure 1 presents the activities of the glycolytic, citric acid cycle, and electron transport system enzymes that were analyzed in the different invertebrate species. As a broad generalization the types of pathways and the flux potentials through

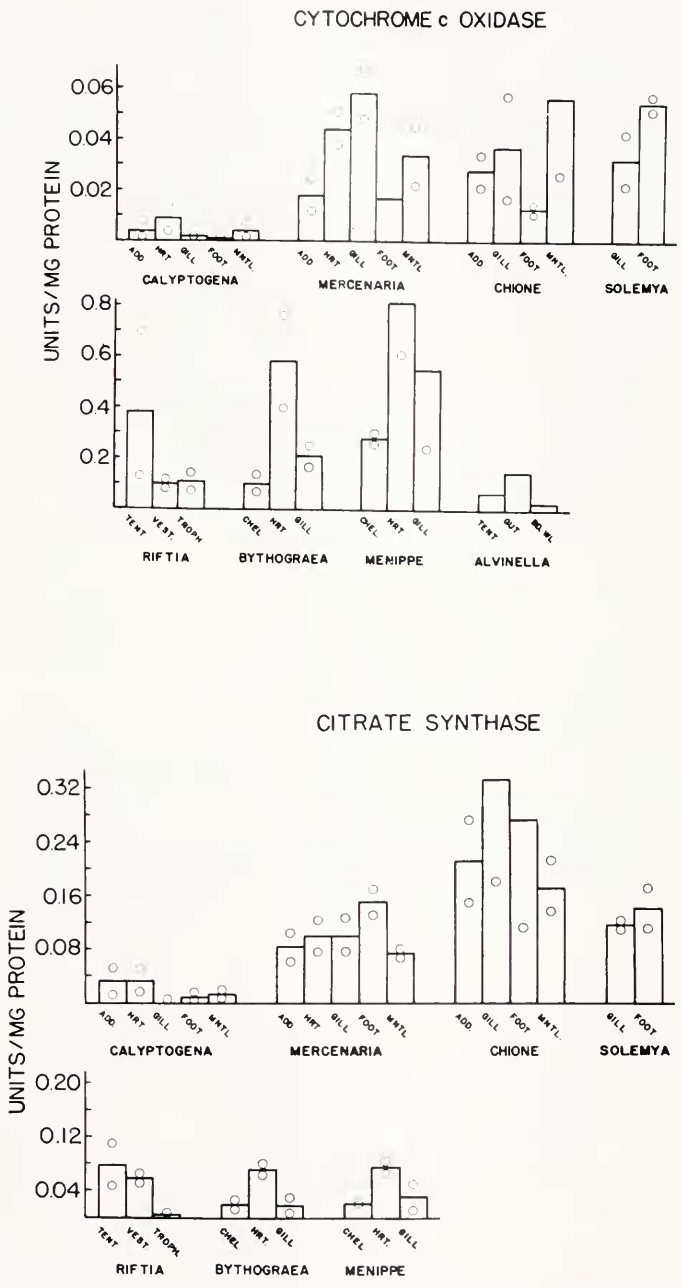

FIGURE 1. Enzymic activities in different tissues of invertebrates from hydrothermal vent and shallow marine habitats. Activities are expressed as international units (μmoles substrate converted to product per minute) per mg protein in the supernatant fractions used as sources of enzyme. The heights of the bars indicate the average values for each tissue; the open circles indicate the measured values. In most cases two individuals of a species were measured. The tissues are abbreviated on the abscissa of each graph as follows: add. (adductor muscle), hrt. (heart), mntl. (mantle), tent. (tentacle), vest. (vestimental muscle), troph. (trophosome), chel. (cheliped), bd. wl. (body wall). The habitats of the species are given in Materials and Methods.

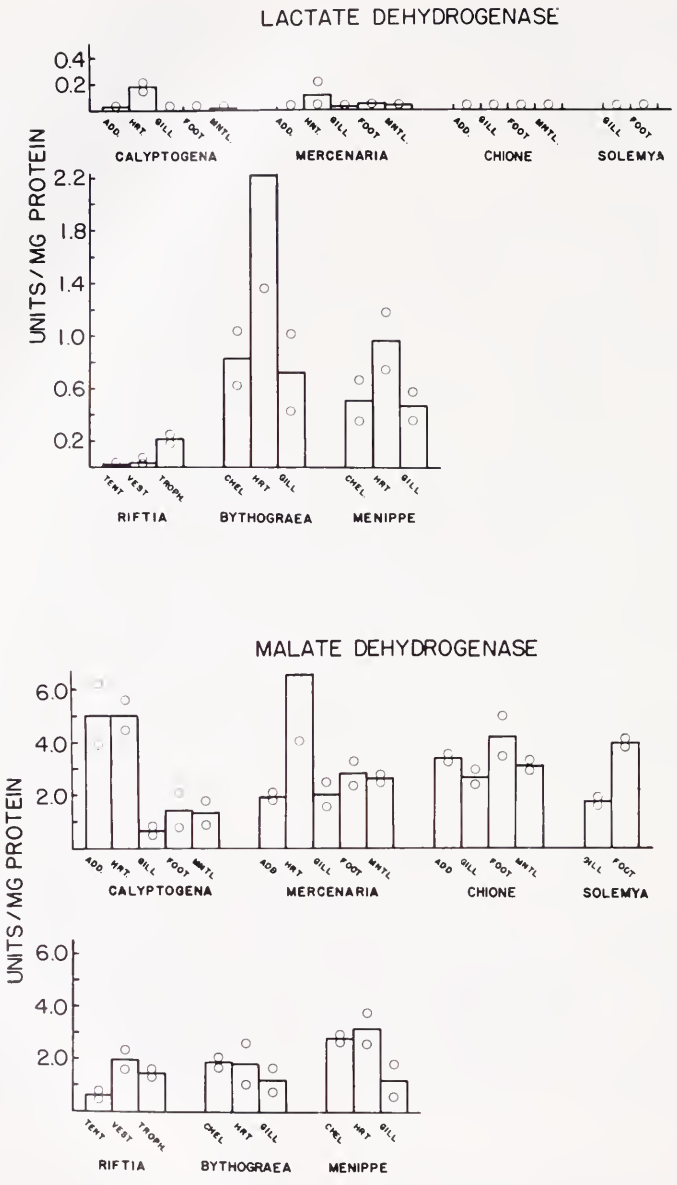

FIGURE 1. (*Continued*)

these pathways appear similar in related vent and non-vent species. For the two crabs, *B. thermydron* and *M. mercenaria,* the types and quantities of enzymic activities found in the tissues studied (cheliped, heart, and gill) were strikingly similar. In both crabs heart tissue displayed the highest aerobic capacity, as judged by activities of CO, a direct indicator of potential for aerobic respiration, and CS, a strong indicator of citric acid cycle flux potential. Activities of these two enzymes were lower in cheliped and gill. The activity of PFK, an indicator of total (aerobic plus

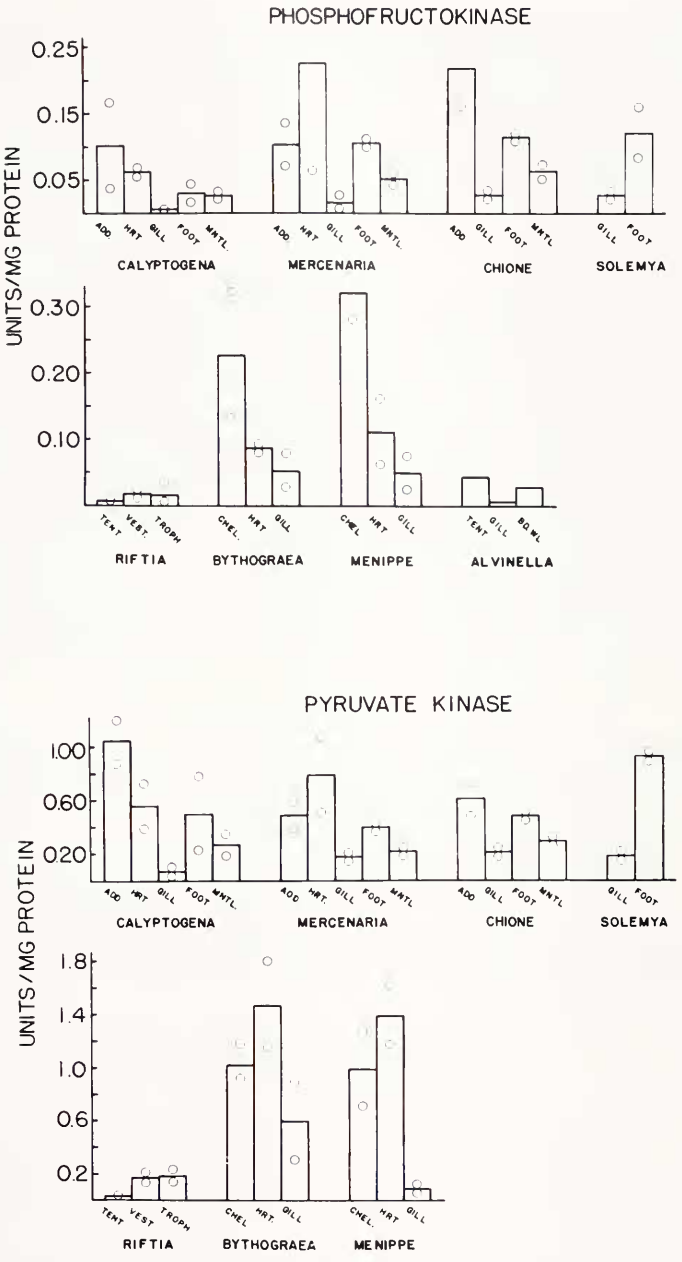

FIGURE 1. (*Continued*)

anaerobic) glycolytic flux potential, was highest in cheliped, as was the activity of PK, another indicator of glycolytic potential. LDH activity, an indicator of a locomotory muscles' capacity for anaerobic glycolysis, also was highest in cheliped. Thus, based on these enzymic activity measurements, there would appear to be a

similar capacity for energy metabolism in the vent crab and subtidal crab, a conclusion that is consistent with oxygen consumption determinations of *B. thermydron* and shallow-living crustaceans under laboratory conditions (Mickel and Childress, 1982).

For the pogonophoran tube worm, *R. pachyptila,* no phylogenetically similar species was available for comparisons. The enzymic activities measured in tissues of *R. pachyptila* do allow, however, for conclusions to be drawn about the abilities of the animal to conduct different types of energy metabolism. The occurrence of CO and CS activities at levels similar to those found in the two crabs suggests that, despite living continuously in the presence of high concentrations of HS^-, *R. pachyptila* is capable of sustaining aerobic respiration. Tentacle (plume) tissue displayed the highest activities of these two enzymes. The tentacle is highly vascularized, and serves as the major site of gas and nutrient exchange between the animal and its environment (Jones, 1981). The aerobic poise of metabolism in tentacle is further suggested by the relatively low levels of activity of the glycolytic enzymes, PFK, PK, and LDH, compared to CO and CS activities. Vestimental muscle, which functions to hold the worm in its tube and to power withdrawal of the tentacle, displayed lower aerobic capacities than tentacle, but it had higher levels of glycolytic activity. The high activities of MDH found in vestimental muscle may be indicative of a high capacity for the type of anaerobic scheme found in many invertebrates, which involves the channeling of phosphoenolpyruvate towards succinate production *via* the intermediates, oxaloacetate, malate, and fumarate (Hochachka, 1980; see Discussion). The trophosome of *R. pachyptila* is a soft, highly vascularized tissue that fills much of the animal's coelom. The trophosome is a complex tissue, containing high densities of bacterial symbionts (up to approximately 10^9 bacteria per g fresh weight; Cavanaugh, 1983; Cavanaugh *et al.,* 1981). Bacterial enzymes may have made the dominant contribution to the enzymic activities measured in trophosome. Like the tentacle and vestimental muscle, trophosome displayed capacities for both glycolytic and electron transport functions.

For the polychaete worm, *A. pompejana,* which grows abundantly on the walls of white smoker chimneys and may be exposed to very high concentrations of sulfide (Desbruyeres and Laubier, 1980; Spiess *et al.,* 1980), limitations in specimen availability precluded making an extensive enzyme survey. However, the Pompeii worm exhibited both PFK and CO activities, suggesting that both glycolysis and aerobic respiration occur in this animal.

Among the four bivalve molluscs we studied, some interesting similarities and differences were noted. The activities of enzymes associated with glycolysis in bivalves, PFK, PK, and MDH, were generally the highest of all enzymic activities, and the capacities for glycolytic flux seemed generally similar in a given tissue among species. LDH activity was very low, in keeping with the fact that MDH, rather than LDH, is the major reaction of glycolytic redox balance in the anaerobic metabolic scheme of bivalves.

Although as a group, bivalves' CO values were considerably lower than those of other species, the most striking difference among the bivalves was the apparently very low capacity for aerobic respiration in *C. magnifica.* CO activities were extremely low in all tissues examined, and were barely measureable in foot. CS activities also were extremely low compared to the other bivalves studied, suggesting that *C. magnifica* has a low capacity for aerobically poised citric acid cycle function. It is noteworthy that another clam from a sulfide-rich habitat, *S. reidi,* which was collected in a sewage outfall habitat where HS^- concentrations of up to 25 m*M* have been measured (J. J. Childress, personal communication) had CO and CS activities

similar to those of *C. undatella* and *M. mercenaria,* two bivalves that do not encounter such high HS^- concentrations in their habitats. Thus a variety of metabolic strategies may be present in bivalves that occur in sulfide-rich environments (See Discussion). In *C. magnifica* and *S. reidi* the gills contain high densities of bacterial endosymbionts (Felbeck *et al.,* 1981; Cavanaugh, 1983; Felbeck, 1983). Thus, as in the case of trophosome tissue of *R. pachyptila,* a significant fraction of the enzymic activities measured in the gills of these two bivalves may be of bacterial origin.

Enzymic activities of the vent zoarcid fish

In keeping with the trends noted for the crustacean and molluscan species examined, the enzymic activities in the vent zoarcid fish were very similar to activities found in many shallow-living fishes. Activities of LDH, PK, and MDH in white muscle of the vent zoarcid were 216 (185, 246), 36 (28, 43), and 41 (19, 62) units per g fresh weight at 10°C, respectively (mean and values for two fish are given). For LDH and PK these activities are compared with data gathered using identical protocols on a number of other marine teleost fishes having different minimal depths of occurrence (Fig. 2). The LDH and PK activities of the vent zoarcid are the highest found for any deep-sea fish, *i.e.,* for any fish having a minimal depth of occurrence greater than approximately 200–300 m, and these activities are within the range noted for many shallow-living, demersal species (cf. Sullivan and Somero, 1980, for discussion of the other species indicated in Fig. 2). MDH shows a similar trend (cf. Sullivan and Somero, 1980).

Sensitivities of cytochrome c oxidase systems to HS^-

Using crude supernatant fractions and, for *R. pachyptila,* partially purified CO, we determined the sensitivities of the CO systems of several animals (Table I). In all cases, half-inhibition (K_i) concentrations of HS^- were in the range of 10^{-9} to 10^{-5} *M*. Even though the CO system of tentacle tissue of *R. pachyptila* appears less sensitive to HS^- than the other CO systems studied (however, see Discussion), in all cases the CO systems of the vent animals were inhibited by HS^- concentrations that were much lower than environmental levels and, in *R. pachyptila,* were vastly lower than the HS^- concentrations found in the animal's blood, where HS^- concentrations up to 1.1 m*M* have been measured (Arp and Childress, 1983). The bases for the interspecific differences in CO sensitivity to HS^-, and possible mechanisms for resistance to poisoning by HS^- are discussed below.

Discussion

The major conclusion resulting from these comparisons of enzymic activities of animals from the hydrothermal vents habitat and shallow marine habitats is that, in almost all cases, the tissues of the vent animals have similar types of energy metabolism pathways, and similar potentials for flux through these pathways, to tissues of shallow-living marine species of similar phylogenetic status. These qualitative and quantitative similarities in the energy metabolism pathways of these two groups of organisms merit discussion in terms of the physical, chemical, and biological characteristics of the hydrothermal vent habitats.

The generally similar activities of the diagnostic enzymes of glycolysis, the citric acid cycle, and electron transport in the tissues of vent animals and shallow-living animals suggest that these two groups of organisms have very similar metabolic rates. Childress and Somero (1979) showed that activities of enzymes of energy

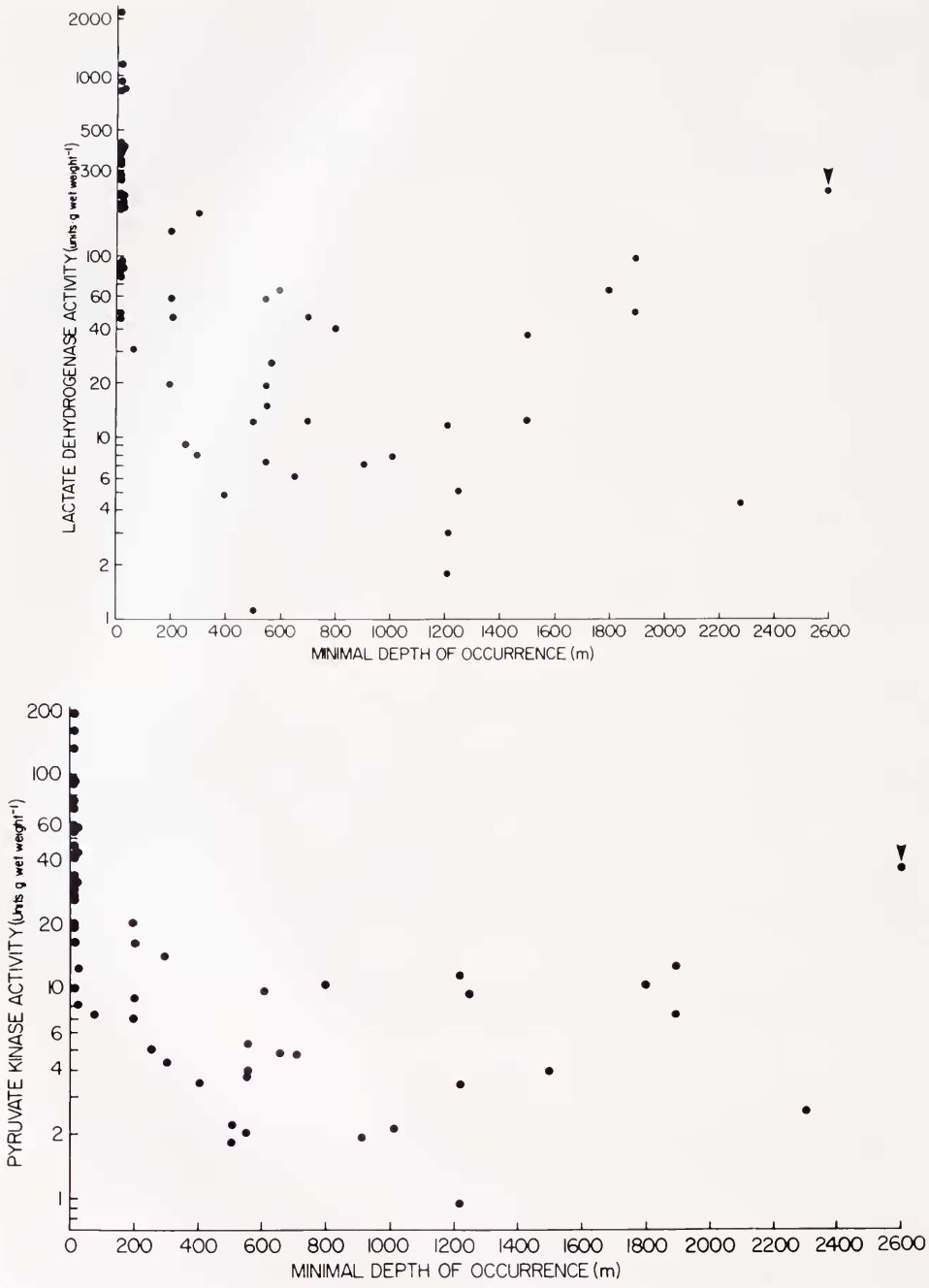

FIGURE 2. Activities of lactate dehydrogenase and pyruvate kinase assayed at 1 atm in white skeletal muscle of marine teleost fishes having different minimal depths of occurrence. Values for the vent zoarcid fish are indicated by the arrow above the point at 2600 m minimal depth of occurrence. Each point represents a different species, and is based on from one to several individuals. Data are from Childress and Somero (1979), Sullivan and Somero (1980), Siebenaller and Somero (1982) and Siebenaller *et al.*, (1982).

metabolism correlate well with rates of oxygen consumption in marine fishes, and the generality of this relationship is further suggested by several other studies of activities of enzymes of energy metabolism in organisms having widely different metabolic capacities (cf. Simon and Robin, 1972; Sugden and Newsholme, 1973; Alp *et al.*, 1976; Zammit *et al.*, 1978; Somero and Childress, 1980; Siebenaller and Somero, 1982). The similarities in amounts of activity of enzymes of energy metabolism in the vent animals and related shallow-living species were noted for the crustaceans, molluscs, and fishes we compared, and although no shallow living pogonophorans were available for comparison (most members of this phylum are endemic to the deep sea; Southward and Southward, 1982), the enzymic activities found in *R. pachyptila* also indicate a substantial capacity for energy metabolism. The vent animals thus contrast sharply with deep-sea animals from non-vent habitats. Animals from non-vent regions in the deep sea have been shown to have extremely low metabolic rates (Childress, 1975; Smith and Hessler, 1974; Smith, 1978; Torres *et al.*, 1979) and very low amounts of activity of enzymes of energy metabolism in their tissues (Fig. 2; Childress and Somero, 1979; Sullivan and Somero, 1980; Siebenaller and Somero, 1982; Siebenaller *et al.*, 1982). For example, the activities of LDH in fish locomotory muscle differ by almost three orders of magnitude between highly active, shallow-living fishes and sluggish deep-sea fishes (Fig. 2).

The finding that animals from the hydrothermal vent habitat have a high potential for energy metabolism is further evidence that the low temperatures and elevated hydrostatic pressures of the deep sea are not, in and of themselves, important factors in selecting for low metabolic rates in deep-sea organisms. At the 21°N site where the vent species used in this study were collected, pressure was approximately 260 atms (depth of 2600 m), and the temperature of the water in the immediate vicinity of the animals was below approximately 20°C and, in almost all cases, was probably within one or two degrees of the ambient bottom water's temperature (near 2°C) (J. J. Childress, personal communication). The Pompeii worm was the only species likely to experience temperatures much above 2–5°C, since this polychaete forms burrows on the sides of white smoker chimneys (Desbruyeres and Laubier, 1980).

Waters issuing from the vents are rich in HS^-, methane, and hydrogen (Edmond *et al.*, 1982), all of which are energy-rich compounds that can be oxidized by chemolithotrophic bacteria. The base of the food chain at the vents is thought to be bacteria, *e.g.*, sulfide-oxidizing chemoautotrophic bacteria, that occur free-living in the sea water (Karl *et al.*, 1980), on the surfaces of rocks and animals, and within certain tissues of *R. pachyptila, C. magnifica,* and the vent mussel (Cavanaugh *et al.*, 1981; Felbeck, 1981; Felbeck *et al.*, 1981; Felbeck and Somero, 1982; Cavanaugh, 1983). The existence of primary production by bacteria at the vents may preclude the vent animals from having to rely significantly on reduced carbon and nitrogen compounds descending from the surface, a conjecture supported by stable carbon and nitrogen isotope ratios (Rau and Hedges, 1979; Rau, 1981a, b; Williams *et al.*, 1981). Although this point remains to be proven, the rates of primary production at the vents may be high enough to allow the vent animals to sustain metabolic rates comparable to those found for animals in food-rich, shallow marine habitats. High metabolic capacities are noted for vent animals containing bacterial endosymbionts (*R. pachyptila* and *C. magnifica*), and for species that graze on bacteria or prey on the vent animals. It bears mentioning that one of the two zoarcid fishes used in this study contained fresh trophosome tissue of *R. pachyptila* in its gut (Somero, personal observations).

TABLE I

Inhibition by HS^- of the cytochrome c oxidase systems of vent and non-vent marine invertebrates

Species [enzyme preparation]	*Inhibition constant* (K_i)([HS^-] yielding 50% inhibition)
Bythograea thermydron	
[heart supernatant]	2.0×10^{-9}
Riftia pachyptila	
[tentacle supernatant]	1.4×10^{-5}
[once acid precipitated]	3.5×10^{-6}
[twice acid precipitated]	1.8×10^{-6}
Mercenaria mercenaria	
[heart supernatant]	1.4×10^{-7}
Menippe mercenaria	
[heart supernatant]	2.0×10^{-7}

In all of the vent animals examined except *C. magnifica* the levels of CO activity present in different tissues suggested a significant capacity for aerobic respiration. The occurrence of the CO system in animals exposed to HS^- concentrations known to be adequate to completely inhibit respiration (Hydrogen Sulfide, 1979; Powell and Somero, 1983) suggest that the vent animals, as well as species like *S. reidi* that live in other sulfide-rich marine habitats, may have evolved mechanisms for prevention of poisoning by HS^- of aerobic respiration. We found no evidence of sulfide-insensitive variants of the CO system in these species. Thus, half-inhibiting concentrations of HS^- for the vent species ranged between 2×10^{-9} *M* (*B. thermydron*) and 1.4×10^{-5} *M* (crude supernatant of tentacle of *R. pachyptila*). Concentrations of HS^- in the vent waters can approach 1 m*M* (Edmond *et al.*, 1982), albeit HS^- concentrations are much lower in the waters immediately surrounding the animals, and blood sulfide levels in *R. pachyptila* of up to 1.1 m*M* have been found (Arp and Childress, 1983). Thus, in the absence of mechanisms for preventing HS^- from coming into contact with the CO system, there would appear to be a strong likelihood that aerobic respiration would be sulfide poisoned in the vent animals. In *R. pachyptila* one possible mechanism for prevention of poisoning of aerobic respiration by HS^- entails essentially quantitative binding of HS^- to blood-borne sulfide binding (transport) proteins (Arp and Childress, 1983; Powell and Somero, 1983). The increase in sensitivity of the CO system of tentacle of *R. pachyptila* to HS^- with successive acid precipitation purification steps (Table I) reflects the removal of these sulfide binding proteins from the system. Thus, even though the CO system of *R. pachyptila* displays a somewhat reduced sensitivity to HS^- compared to the other CO systems studied, we predict that the inherent sensitivities of completely purified CO systems from all of these animals would be essentially equal.

In addition to sulfide binding proteins that may function both in protection of respiration and in sulfide transport to bacterial endosymbionts (Arp and Childress, 1983), systems for oxidizing HS^- to less toxic, or non-toxic, sulfur metabolites may be present in the cells of animals from sulfide-rich environments. For example, we have found high activities of these types of reactions in foot of *S. reidi* (Powell and Somero, in prep.). In assays of CO activity that use crude supernatant fractions that contain sulfide oxidizing enzyme systems as well as CO activity, the K_i value obtained may be artifactually high due to the removal of HS^- from the assay solution by the sulfide oxidizing system. Thus, the K_i values listed in Table I should be viewed as upper limits to the K_i values that would be found in the absence of sulfide binding

proteins or sulfide oxidizing systems, both of which can effectively reduce the amount of free HS^- present in the assay medium.

Calyptogena magnifica was the only vent species to show marked differences in metabolic potentials relative to the shallow-living comparison species. Although tissues of *C. magnifica* had activities of PFK, PK, and MDH that were comparable to, and often higher than, the corresponding activities in the other bivalve molluscs examined, levels of CS and CO were extremely low in the vent clam. Thus, the enzyme profiles of *C. magnifica* are suggestive of a very high reliance on anaerobic metabolism. In certain marine bivalves a substantial fraction of energy metabolism occurs via anaerobic pathways even in the presence of oxygen (DeZwann and Wijsman, 1976). The diagnostic enzymes for high potentials for the types of anaerobic schemes common in marine bivalves include MDH, the enzyme showing the highest activity in adductor and heart muscle of *C. magnifica*. The basis for this species' reliance on anaerobic metabolism may be the nature of the microhabitat in which the clam is found. *Calyptogena magnifica* at the 21°N study site were almost invariably found along cracks in the basaltic seafloor through which sulfide-rich waters issued (personal observations). The large foot of the clam was sometimes extended deeply into the crack, and thus was exposed to high concentrations of HS^-. The steady flux of high quantities of HS^- into the clam may preclude the possibility of detoxifying HS^- by the mechanisms discussed above, and without the means for preventing contact between HS^- and the CO system, aerobic respiration is not possible. It is important to point out, however, that *C. magnifica* does "respire" in the sense that the intact symbiosis consumes oxygen at an appreciable rate (Kenneth L. Smith, Jr., personal communication), as has recently been reported for *Calyptogena pacifica* (Childress and Mickel, 1982), which also harbors bacterial endosymbionts in its gills (Felbeck *et al.*, 1981). As Childress and Mickel (1982) emphasize, caution must be exercised in attempts to attribute specific fractions of oxygen uptake to the animal's tissues, on the one hand, and the sulfide oxidizing bacterial endosymbionts, on the other. The very low CO activities found in *C. magnifica* suggest that by far the larger share of oxygen consumption by the intact symbiosis may be due to the sulfide oxidizing activities of the endosymbionts.

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